Synthesis of 3-amino-2-alkenoate

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Abstract

The main aim of the project is to synthesize an isomerically pure sample of 3-amino-2-alkenoate, a useful β-amino acid derivative that is a precursor to molecules with medicinal properties. We successfully made the product from the reaction of ethyl acetoacetate and ammonium acetate dissolved in methanol. We also attempted to synthesize 3-pyrrole-2-alkenoate from the reaction of our product and 2,5-dimethoxytetrahydrofuran. The products of both reactions were chracterized by means of ^{1}H nuclear magnetic resonance spectroscopy.

1 Introduction

Organic chemistry research has revealed the promising applications of β-amino acids and their derivatives, encouraging further study of their properties and methods of synthesis. β-amino acids rarely occur in nature, but they do appear in free form or in active peptides [9]. β-amino acids have one additional carbon in the parent chain compared to the more common α-amino acids used to make most polypeptides. Therefore, they have different properties and can be used to create new classes of organic molecules. For example, peptides composed of β-amino acids are resistant to decomposition by enzymatic activity [6], whereas α-peptides are not. There is also evidence that β-peptides may fold in similar ways to α-peptides under certain conditions, specifically when nearby monomers have weak intermolecular forces between them [5]. They have also been shown to form secondary structures similar to those of α-peptides [10]. Since β-peptides may be useful to the human body but are not broken down by digestion, they are potentially a novel class of medicinal molecules. In addition to peptides, β-amino acid derivatives can form other useful molecules. For example, β-amino-α-hydroxy acids can be used to produce taxol, a treatment for tumors; bestatin, an immune system modifier; and oligopeptides that can be used as sedatives [3]. Also, β-amino acids are used to produce β-lactams, a class of antibiotics that includes penicillin [1].

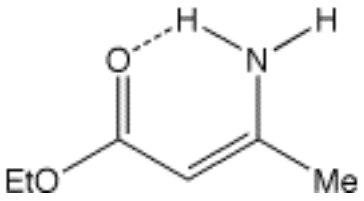


Figure 1: 3-amino-2-alkenoate

In this paper we explore an approach to the synthesis of 3-amino-2-alkenoate, a useful β-amino acid derivative (Figure 1). Using ethyl acetoacetate, a β-keto ester, as starting material, we seek a pure sample containing only the shown isomer. We also present a reaction mechanism that is plausible given the chemistry of the reaction. We also share our work on a method for the formation of the vinyl pyrrole of the 3-amino-2-alkenoate.

Thin-layer chromatography (TLC) and gas chromatography (GC) were used to track the progress of the reaction and verify that it had gone to completion. Flash chromatography (column chromatography) was used to purify the sample and to separate out components. Nuclear magnetic resonance (NMR) spectroscopy was conducted to ensure the fidelity of the product. ^{1}H NMR signals vary based on the chemical environments of protons in the molecule and therefore can be used to infer a structure.

Goals of synthesis include removal of impurities,

optimization of yield, separation of isomers, and verification of product identity. β-amino acid derivative samples with isomeric purity are important in forming arrangement-dependent molecules because alternative isomers may not react identically [6]. Here, we are concerned with the cis-trans isomeric disparity that occurs with double bonds. We seek the cis isomer, with the amino group interacting with the carbonyl group. Cis-trans isomers often either are in equilibrium with each other or are produced coexisting in the same sample. One efficient method for isomeric isolation is to determine a convenient step of the synthesis process where the isomers can be separated via chromatography, because the isomers of some molecules have greater differences in physical properties than those of others.

2 Experimental Section

2.1 General Procedures

All reactions were performed in flame-dried round bottom flasks fitted with rubber septa under a positive pressure of argon. Air- and moisture-sensitive liquids and solutions were transferred via sterile syringe. Analytical thin-layer chromatography was performed using glass plates precoated with 0.25 mm 230–400 mesh silica gel impregnated with a fluorescent indicator (254 nm). Thin-layer chromatography plates were visualized by exposure to ultraviolet light and to an indicator solution of *p*-anisaldehyde followed by heating on a hot plate. Organic solutions were concentrated by rotary evaporation at reduced pressures and high temperatures.

2.2 Instrumentation

Proton NMR spectra were recorded with a Mercury Varian (300 MHz or 500 MHz). Chemical shifts for protons are reported in parts per million downfield from tetramethylsilane and are referenced to residual protium in deuterated chloroform or benzene. NMR data are organized as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, coupling constants in Hertz, and assignment.

2.3 Materials

Ethyl acetoacetate was purchased from Aldrich and distilled within the laboratory. The methanol and ammonium acetate were purchased from Fisher and Alfa Aesar respectively. 2,5-dimethoxytetrahydrofuran, 10-camphorsulfonic acid, and all organic solvents were purchased from Aldrich.

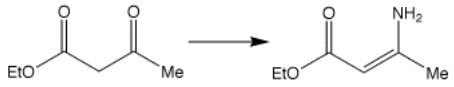


Figure 2: Ethyl acetoacetate and 3-amino-2-alkenoate

2.4 3-amino-2-alkenoate

The reaction to produce the β-amino acid derivative (Figure 2) was conducted as follows. A 250 mL round-bottom flask (RBF) with a magnetic stir bar was flame-dried using a propane torch. Methanol (150. mL) was poured into the RBF along with solid ammonium acetate (46.2 g). The flask was then covered with a septum and evacuated and flushed with argon three times and then put under positive argon pressure. 15.3 mL of ethyl acetoacetate was syringed into the flask. The flask was placed over a magnetic spinner and stirred. Theoretical yield was calculated to be 15.5 grams.

Within an hour after the reaction had been begun, a 0.1 mL sample was taken from the flask and diluted with dichloromethane in order to execute a TLC (solvent: 25% EtOAc/hexanes) and a GC (maximum retention time: 15 minutes). Both demonstrated that the reaction was proceeding rapidly. The reaction was continued for another 15 hours before another sample determined that the reaction was complete.

The flask was then placed on a rotary evaporator to evaporate excess solvent. Water was observed on the edges of the flask. The product was then diluted with 300. mL of dichloromethane, which produced a solid white precipitate. The precipitate was vacuum filtered and then rinsed with $2 \times 300.$ mL of dichloromethane. The filtrate was poured into a separatory funnel and washed first with distilled water and then with brine. Because dichloromethane is denser than water, the organic layer and therefore the product sank to the bottom. The organic layer was collected in an Erlenmeyer flask and dried over anhydrous sodium sulfate. The product was then vacuum-filtered again and then concentrated in a 1000 mL RBF using a rotary evaporator. After the solvent

had evaporated, the yield of the crude 3-amino-2-alkenoate was 15.0 grams.

A 5 cm-wide chromatography column was prepared as described by McMurry [8]. Approximately 1500 mL of solvent was made from 10% EtOAc/hexanes. Anhydrous sodium sulfate was poured into the bottom followed by 8 cm of compressed silica gel. After more sodium sulfate was added to the top, the product was loaded onto the column and was allowed to submerge into the silica before the rest of the column was filled with solvent. 30 mL fractions were taken and a TLC was done on each. Fractions containing the material that matched the characteristics of the product were pooled into a preweighed RBF. Fractions containing other material were pooled separately. The RBF was placed on a rotary evaporator to obtain the product. The final yield of the pure 3-amino-2-alkenoate was 12.0 grams (77.4%).

NMR spectroscopies were taken of the 3-amino-2-alkenoate. The placement of the hydrogens in the molecule showed that both the cis and trans forms were present, most likely in rapid equilibrium with each other. Because of the fast cis-trans isomerization of 3-amino-2-alkenoate, it was unlikely that the isomers would be separable. Therefore, the combined isomers were reacted to synthesize 3-pyrrole-2-alkenoate considering its slower isomerization.

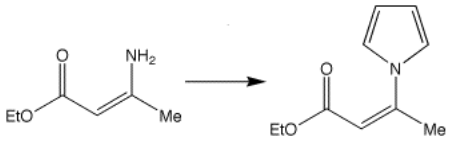


Figure 3: 3-amino-2-alkenoate and 3-pyrrole-2-alkenoate

2.5 The Vinyl Pyrrole

The synthesis of 3-pyrrole-2-alkenoate (Figure 3) was attempted. 500.2 mg of the 3-amino-2-alkenoate product of the previous reaction was weighed out and placed in a 100 mL round-bottom flask. 90.02 mg of solid 10-camphorsulfonic acid was added, and then the flask was covered with a septum. It was subsequently placed under vacuum and flushed with argon three times before being put under positive argon pressure. 38.7 mL of THF was syringed into the flask in order to make the molarity of 3-amino-2-alkenoate 0.1 M. Then 0.500 mL of 2,5-dimethoxytetrahydrofuran was syringed to start the reaction. Theoretical yield was calculated to be 694 mg.

Within one hour, the reaction mixture was sampled for TLC and GC. Both indicated the reaction was complete. Two main compounds seem to have been formed. We hypothesized that they were the cis and trans isomers of the vinyl pyrrole. The molecules stained and separated in the same way as the TLC standard, but the retention times were drastically different from the GC standard for the vinyl pyrrole.

The reaction mixture was worked up by washing with 25 mL water and 50 mL ethyl acetate and separation in a separatory funnel. Any extra product remaining in the aqueous layer was extracted by washing with 3×25 mL ethyl acetate in the separatory funnel. All organic layers were then combined and dried over sodium sulfate. The product was then filtered by vacuum to remove sodium sulfate and other solid impurities. Solvent was then evaporated under reduced pressure.

Flash chromatography was performed on the product using a 3 cm-wide column and 10 mL fractions. The solvent system was 10% EtOAc/hexanes. One pool provided one of the major products, another provided a product not previously seen and that appeared to be starting material, and no pool provided the second major product. Impure fractions were pooled together to form a final pool, which was subjected to a second flash chromatography after being concentrated. The second column utilized 3% EtOAc/hexanes as solvent in order to induce greater separation. 482 mg of product was recovered.

NMR spectroscopy indicated that neither of the primary products of the reaction were the correct product, implying that unwanted hydrolysis had occurred. A second reaction, using acetic acid instead of 10-camphorsulfonic acid, gave the same GC results; presumably the same compounds were formed.

3 Results

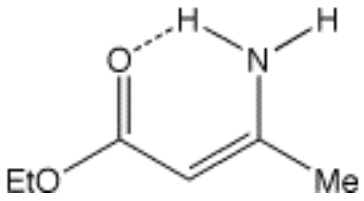
3.1 3-amino-2-alkenoate

The ethyl acetoacetate and the product were both UV-active and had virtually the same Rf (retention factor) values. In 25% EtOAc/hexanes, the Rf value was 0.31. Anisaldehyde caused the starting material to stain white surrounded by red. The product stained red. These agreed with chromatographic standards, so TLC indicated that the product was

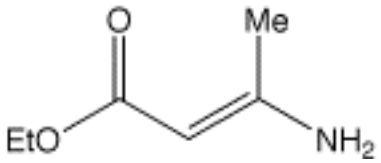
3-amino-2-alkenoate.

The retention times of ethyl acetoacetate were 1.624 and 2.269 minutes. Ethyl acetoacetate does not behave well in GC procedures, changing between the enol and ketone forms and creating two retention times. The retention time of the product was 4.330 minutes. This agreed with chromatographic standards, so GC indicated that the correct product had been formed.

NMR results are as follows.



^{1}H NMR (500 MHz, $CDCl_3$, 20 °C): 7.93 (bs, 1H, N**H**), 4.54 (bs, 1H, N**H**), 4.52 (s, 1H, C=C**H**), 4.11 (q, 2H, J= 7 Hz, $CH_3C\mathbf{H_2}$), 1.91 (s, 3H, $C\mathbf{H_3}C$=C), 1.26 (t, 3H, J= 7 Hz, $C\mathbf{H_3}CH_2$).



^{1}H NMR (500 MHz, C_6D_6, 20 °C): 5.00 (s, 1H, C=C**H**), 4.21 (q, 2H, J= 7 Hz, $CH_3C\mathbf{H_2}$), 2.27 (s, 3H, $C\mathbf{H_3}$C=C), 1.28 (t, 3H, J= 7 Hz, $C\mathbf{H_3}CH_2$), NH_2 protons not observed.

NMR showed that both cis-trans isomers were present in the sample. This was inferred from the two different peaks for the protons in the methyl group and two different peaks for the protons in the amino group. The hydrogen bonding of the amino group to the nearby carbonyl group produced the noticeable difference.

3.2 3-pyrrole-2-alkenoate

The products of the attempted synthesis of the vinyl pyrrole were not UV-active and had Rf values of 0.31 and 0.41, respectively. These agreed with chromatographic standards for the two cis-trans isomers of 3-pyrrole-2-alkenoate, indicating that the correct products had been formed.

The retention times of the products of the attempted synthesis of 3-pyrrole-2-alkenoate had retention times of 2.307 and 2.451 minutes, respectively. These disagreed with the chromatographic standards of approximately 5 minutes. These products were much more volatile than the desired products, demonstrating that the incorrect products had been formed.

NMR results showed that the incorrect products had formed.

4 The Mechanism

A reaction mechanism for the formation of 3-amino-2-alkenoate was proposed (Figure 4). Ammonium acetate is in equilibrium with ammonia and acetic acid. Even though the salt is favored in the equilibrium, the ammonia and acetic acid can still react in the mechanism of the formation of 3-amino-2-alkenoate. The acetic acid protonates **1** because of the high density of electrons around the electronegative oxygen. The extra proton further increases the tendency for electrons to be drawn towards the oxygen, making the carbon nearest to it electron-deficient. This gives the nucleophilic ammonia the ability to donate electrons to the carbon to form **3**. The acetate ion, attracted by charge to the amino group, then pulls off a hydrogen to regenerate acetic acid. Once again, the acetic acid protonates the oxygen due to the oxygen's effective negative charge. Once **5** is achieved, the protonated oxygen makes up a very likely leaving group, and is released as water, taking the carbon-oxygen bond electrons with it. The lone-pair electrons on the amino group are forced to bond with the carbon to stabilize the compound, thus forming **6**. Because the amino group is positively charged, it attracts the acetate anion and gives up its proton to form **7**. In the final step, tautomerization occurs as a hydrogen is transferred from within the molecule to the amino group, causing a rearrangement of bonds. Because of the stability of the hexagonal ring, 3-amino-2-alkenoate is unlikely to participate in the reverse reaction.

Overall, the acetic acid is not consumed and is therefore a catalyst. The ammonia, however, reacts with the ethyl acetoacetate. Water is formed as a product, which was observed in the reaction. The reaction does not go to completion quickly; it takes several hours to finish, which is perhaps due to the reversibility of each step of the mechanism. The mechanism fits the observations we made.

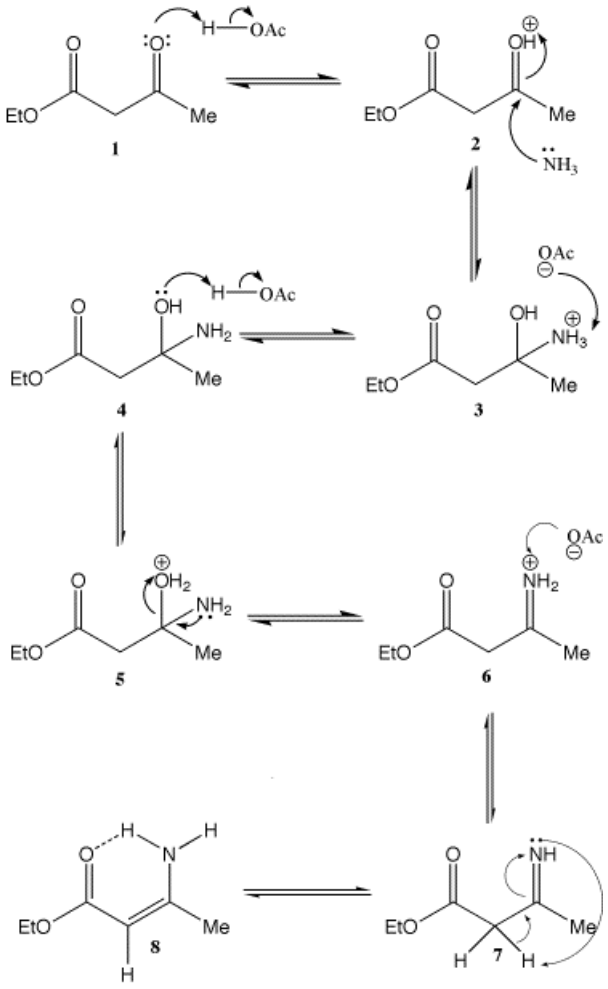


Figure 4: Proposed mechanism for the production of 3-amino-2-alkenoate

5 Future Studies

Further investigation into the viability of the method of synthesizing 3-pyrrole-2-alkenoate from 3-amino-2-alkenoate is needed. Research into the production of β-amino acids from the molecules synthesized here could provide this work with numerous medicinal applications. Other pathways from ethyl acetoacetate to 3-pyrrole-2-alkenoate should also be studied (Figure 5).

6 Conclusion

We have found that the synthesis of 3-amino-2-alkenoate from ethyl acetoacetate is a viable and moderately efficient method and presented a mechanism explaning the reaction's success. This product was purified and has been isolated with 77.4% yield. We have also found, however, that one method of producing 3-pyrrole-2-alkenoate from 3-amino-2-alkenoate is not viable. Our findings build onto the growing knowledge pool of how to best synthesize β-amino acid derivatives for eventual commercial or industrial use.

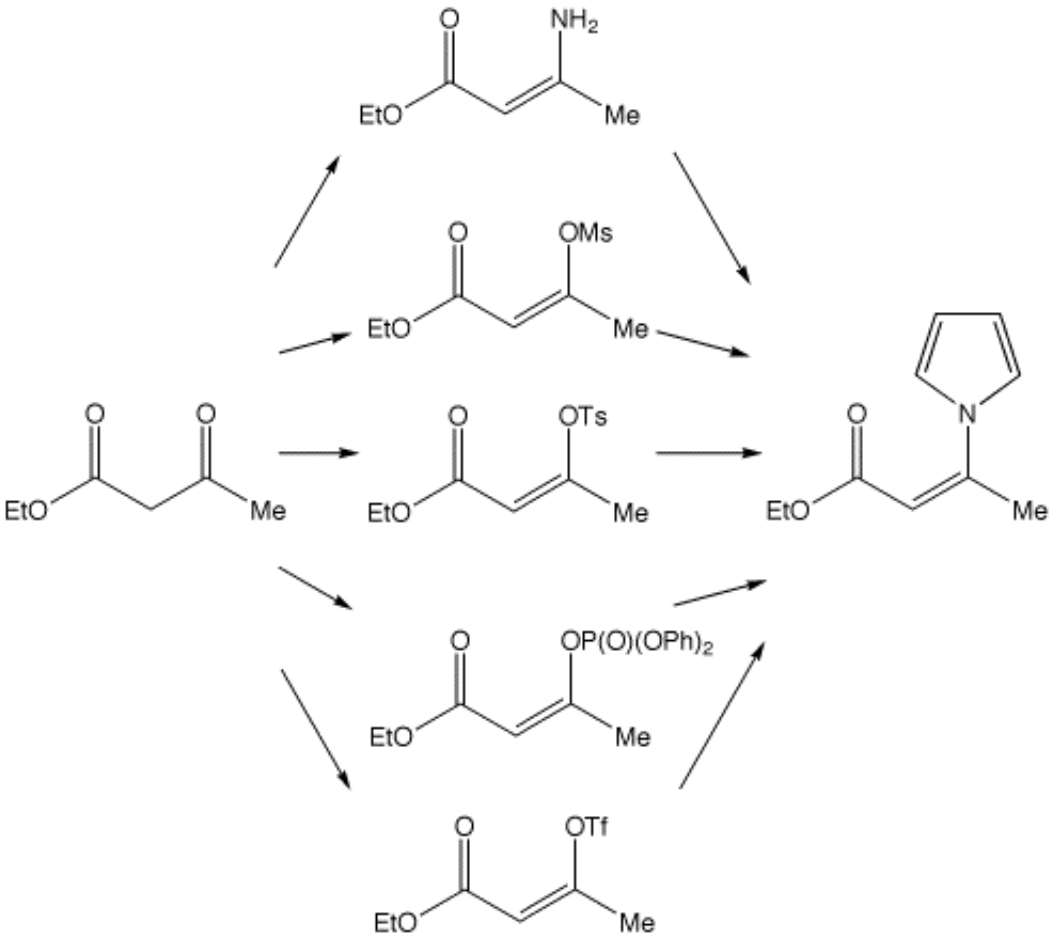


Figure 5: Other pathways currently being explored

7 Acknowledgments

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